REMARKS

A check for a two month extension of time accompanies this response. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition. A supplemental Information Disclosure Statement is sent same day under separate cover.

Claims 1-33 and 42-44 are pending. Claims 1, 9, 16, 17, 22 and 23 are amended to clarify particulars of the claim language. For example, claims 1, 9, 22 and 23 are amended to clarify that the predetermined property and the activity referred to in the claims is the same and that the predetermined property is a chemical, physical or biological property of a target protein. Basis for these amendments can be found, for example at page 9, lines 6-8 and in the claims as originally filed. Claim 1 is also amended to clarify that each hit contains a mutation designated as a hit position. Basis for this amendment can be found, for example at page 34, lines 8-20. The amendment to claim 16 finds basis for example, at page 35, lines 1-9 and in the claim as originally filed. The amendment to claim 17 finds basis, for example, at page 34, line 26 to page 35, line 26 and in claim 17 as originally filed. Inadvertent typographical errors pointed to in the Office Action at pages 4 and 6 are corrected herein. No new matter is added.

I. Objections to the disclosure

The Office Action sets forth a number of objections to the specification for typographical errors. These objections are addressed and obviated as follows:

1. "nInternational" at page 4, line 13 – Applicant submitted a Preliminary amendment mailed March 1, 2002 correcting this typographical error.

2, "PCT n" at page 4, line 21 and page 6, line 2 is corrected herein (see page 2 of this paper).

3. "mehods that adapt" at page 8, line 24 - Applicant submitted a Preliminary amendment mailed March 1, 2002 correcting this typographical error.

4. "Gen ration of" page 30, line 3 – Applicant has reviewed the hard-copy of the application as filed and is unable to find this typographical error therein. Applicant respectfully requests clarification and confirmation that this error was not created perhaps in the USPTO electronic scanning process of the hard-copy application filed. A hard-copy of this page as originally filed is enclosed herewith.

5. "dis very f protein variants a th amino" at page 31, lines 1-2 - Applicant has reviewed the hard-copy of the application as filed and is unable to find this typographical error therein. Applicant respectfully requests clarification and confirmation that this error was not created perhaps in the USPTO electronic scanning process of the hard-copy application filed. A hard-copy of this page as originally filed is enclosed herewith.

Therefore, in light of the remarks above, Applicant respectfully submits that the objections to the specification are obviated.

II. Provisional Obviousness-Type Double Patenting

Claims 1, 5-9 and 27 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1, 4, 13, 14, 17 and 18 of copending U.S. Application Serial No. 10/375,192. It is alleged that the claims of the two applications are not patentably distinct from each other because they have overlapping embodiments. This rejection is respectfully traversed.

Relevant law

Obvious-type double patenting signifies that the difference between a first-patented invention and a variant involves only an unpatentable difference, such that grant of the second patent would extend the right of exclusivity conferred by the first patent. Comparison can be made only with what is claimed in the earlier patent, paying careful attention to the rules of claim interpretation to determine what invention a claim defines and not looking to the claim for anything that happens to be mentioned in it as though it were a prior art reference. A fundamental rule of claim construction requires that what is claimed is what is defined by the claims taken as a whole, every claim limitation (each step) is material. General Foods Corp. v. Studiengesellschaft Kohle mbH, 23 USPQ 1839 (Fed. Cir. 1992). *The disclosure of a patent cited in support of a double patenting rejection cannot be used as though it were prior art even where the disclosure is found in the claims.*

Obviousness-type double-patenting has not been found in instances in which the claims at issue do not embrace the prior patent compounds and/or the claims in the prior patent do not suggest any modification that would have produced the claimed compounds in the patent or application at issue (see, e.g., Ortho Pharmaceutical Corp v. Smith, 22 USPQ2d 1119 (Fed. Cir. 1992)), in which obvious-type double patenting was not found in an instance in which the claims in the patent at suit were directed to compounds that did not encompass, structurally, the compounds claimed in the prior patents, and the compounds claimed in the prior patents did not suggest a modification of those compounds to produce compounds

claimed in the patent at suit. Thus, obvious-type patenting does not exist if the claims at issue do not encompass the claimed subject matter in the copending application, and, the claims in the copending application do not suggest a modification to produce the claims in the subject application

Analysis

Applicant respectfully submits that there is no obvious-type double patenting as between the claims of the instant application and the claims of copending U.S. Application serial No. 10/375,192.

Comparison of the instant claims and claim 1 of U.S. Appln. Serial No. 10/375,192

Claim 1 of the instant application recites the steps of:

(a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein;

(b) introducing each set of nucleic acid molecules into host cells and expressing the encoded protein, wherein the host cells are present in an addressable array:

(c) individually screening the sets of encoded proteins, whereby one or more proteins that have a predetermined property that differs from the target protein are identified, wherein each such protein is designated a hit and each hit contains a mutation designated a hit position and the predetermined property is selected from among chemical, physical and biological property of the target protein.

Instant claim 1 and claims 5-9 and 27 that depend from claim 1, and incorporate all of its limitations, are directed to a method for identification of modified target proteins by producing populations of nucleic acid molecules encoding the modified proteins, expressing the modified proteins from the nucleic acid molecules in host cells and screening the modified proteins for a property that differs from the unmodified target protein. The product of the instantly claimed methods are modified target proteins. In step c) sets of encoded proteins are screened.

Claim 1 of of Application No. 10/375,192 recites a method for the production of a *nucleic acid* molecule having a predetermined property. The method includes the steps of:

(a) producing a population of sets of target functional nucleic acid molecules that each comprise a target modified functional sequence of nucleotides;

(b) introducing each set of nucleic acid molecules into host cells and expressing a protein whose expression is modulated or regulated by the target functional sequence of nucleotides, wherein the host cells are present in an addressable collection;

(c) individually screening the sets of encoded proteins to identify the target functional nucleic acid molecules whose activity is altered, wherein each such target functional nucleic acid is designated a hit.

Thus, claim 1 and dependent claims 13, 14, 17 and 18 of U.S. Application Serial No. 10/375,192 are directed to the production of modified target functional sequences of nucleotides. The claimed methods of U.S. Application Serial No. 10/375,192 include a step of introducing the modified target functional sequence of nucleotides into host cells and expressing a protein whose expression is modulated or regulated by the target sequence of nucleotides to identify target functional sequence of nucleotides with altered activity. **The claimed methods of Application No. 10/375,192 produce modified target functional sequence of nucleotides, not modified proteins.** The proteins are screened to assess differences in expression of the encoding nucleic acid molecule not to assess differences in properties of a target protein. Additionally, the modified target functional sequence of nucleotides identified by the methods of Application No. 10/375,192 *modulate or regulate* a protein; they do not encode a modified protein. Hence, the methods are directed to the identification of completely different products.

Further, although the Office Action alleges that the methods steps of the instant application encompass the steps of the method of Application No. 10/375,192, such allegation is mistaken. Claim 1 of the instant application recites the steps of:

> (a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein;
>
> (b) introducing each set of nucleic acid molecules into host cells and **expressing the encoded protein,** wherein the host cells are present in an addressable array:
>
> (c) individually screening the sets of encoded proteins, whereby **one or more proteins that have a predetermined property that differs from the target protein are identified**, wherein each such protein is designated a hit and each hit contains a hit position and the predetermined property is selected from among chemical, physical and biological property of the target protein.

Claim 1 of of Application No. 10/375,192 recites a method for the production of a molecule, having a predetermined property that includes the steps of:

> (a) producing a population of sets of target functional nucleic acid molecules that each comprise a target modified functional sequence of nucleotides;

(b) introducing each set of nucleic acid molecules into host cells and **expressing a protein whose expression is modulated or regulated by** the target functional sequence of nucleotides, wherein the host cells are present in an addressable collection;

(c) individually screening the sets of encoded **proteins to identify the target functional nucleic acid molecules** whose activity is altered, wherein each such target functional nucleic acid is designated a hit.

The steps of the method of instant claim 1 do not encompass the steps of the method of claim 1 of Application No. 10/375,192. First, the claimed methods of U.S. Application Serial No. 10/375,192 do not include a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein. The claimed methods of U.S. Application Serial No. 10/375,192 include a step of producing populations of target modified functional sequence of nucleotides; these target modified functional sequence of nucleotides are not designed to encode modified proteins but encode modifications in the regulatory or other functional sequences of nucleotides. Second, the methods of instant claim 1 include introducing sets of nucleic acid molecules that express the modified proteins. In contrast, the claimed methods of Application Serial No. 10/375,192 do not express a protein that is encoded by the modified functional sequence of nucleic acids. These methods express a protein whose expression that is *regulated or modulated* by the modified functional sequence of nucleic acids (underlined for emphasis above). Finally, the methods of instant claim 1 include a step of identifying proteins that have a predetermined property that differs from a target protein. In contrast, the method of claim 1 of U.S. Application Serial No. 10/375,192 includes a step of identifying modified target functional nucleic acid molecules with altered activity. Hence, the methods of the instant application result in production and identification of modified proteins with different activity from a target protein; whereas the methods of U.S. Application Serial No. 10/375,192 include a step of identifying modified target functional sequences of nucleotides. Claim 1 and claims dependent thereon of the instant application and claim 1 and claims dependent thereon of copending Application No. 10/375,192 are directed to different subject matter. Thus, neither set of claims from each application embrace or overlap the other. Therefore, there is no obviousness-type double patenting as between the applications.

Comparison of the instant claim 1 and claim 4 of Application No. 10/375,192

The analysis of the distinctions between instant claim 1 and claim 4 of U. S. Application Serial No. 10/375,192 is similar to the above analysis. Claim 4 of U.S.

Application Serial No. 10/375,192 recites a method for the production of a functional nucleic acid molecule having a predetermined property that includes the steps of:

> (a) producing a population of sets of modified nucleic acid molecules that encode modified forms of a target functional nucleic acid, wherein **each modified functional nucleic acid molecule is operably associated with a nucleic acid region encoding a reporter;**
>
> (b) introducing each set of nucleic acid molecules into host **cells under conditions that express a reporter** when using a wild-type functional nucleic acid region, wherein the host cells are present in an addressable collection;
>
> (c) individually screening the sets of nucleic acid molecules encoding reporter proteins to identify one or more target modified functional nucleic acid regions that has activity that differs from the unmodified functional nucleic acid molecule, wherein each such molecule is designated a hit.

This claim is directed to producing modified forms of target functional nucleic acids that are each operably associated with a nucleic acid region encoding a reporter and screening these nucleic acids to identify target modified functional nucleic acid regions that have activity that differs from the unmodified functional nucleic acid molecule.

Hence, claim 4 differs from the claims of the instant application more than claim 1 and dependent claims analyzed above. Claim 4 is directed to methods for producing and screening modified functional nucleic acids, such as modified promoters and regulatory regions. Claim 4 is directed to a method that includes a step of identifying a product completely different from the product identified by a different method in instant claims. For example, unlike claim 1 of the instant application claim 4 of U.S. Application Serial No. 10/375,192, does not include a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein. Claim 4 includes a step of producing populations of target modified functional sequences of nucleotides. Second, the methods of instant claim 1 include introducing sets of nucleic acid molecules that express the modified proteins. In contrast, the method of claim 4 of Application No. 10/375,192 does not include expressing a protein that is encoded by the modified functional sequence of nucleic acids. In the method of claim 4, a reporter is operably linked to modified functional nucleic acid. It is the reporter gene that is expressed (underlined above in claim 4). The expressed reporter protein itself, however, is not modified. Only the functional nucleic acid molecule is modified and it is not expressed. Finally, the methods of instant claim 1 include a step of identify proteins that have a predetermined property that differs from the target protein. In contrast, the method of claim 4 of U.S. Application Serial No. 10/375,192 includes a step of identifying modified target functional nucleic acid molecules that differ from the unmodified

functional nucleic acid molecule. Hence, the methods of the instant application identify modified proteins with different activity, while the methods of claim 4 of application Serial No. 10/375,192 are directed to identifying modified target functional sequences of nucleotides. Since the claim 1 and claims dependent thereon of the instant application and claim 4 and claims dependent thereon of copending U.S. Application Serial No. 10/375,192 are directed to different subject matter, there is no overlap in subject matter nor does one claim embrace a claim in the copending application. Therefore, there is no obviousness-type double patenting as between any of the claims pending in the instant application and the copending application.

III. Rejection of Claims Under 35 U.S.C. §112, second paragraph

Claims 1-33 are rejected as being indefinite. Each of the rejections in particular is addressed in detail below. In light of the amendments herein and the remarks below, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claims 1-33 are rejected as indefinite for failing to recite a final process step that agrees with the preamble. Further, it is alleged that it is unclear if the "activity that differs from the target protein is intended to be the activity of the "predetermined property."

Claim 1 is amended such that the preamble and the final step of the method recite identifying a polypeptide that differs in a predetermined property from a target protein. In addition, the claim recites a "predetermined property" throughout the claim, clarifying that the proteins are screened for a predetermined property that differs from the target. Hence, Applicant respectfully submits that the metes and bounds of the claims are clear.

Claims 1 and 9 are alleged to be vague in reciting the limitation "to identify one or more proteins that have activity that differs from the target protein." It is alleged that "activity" is unclear.

Claim 1 is amended herein to render it clear that the proteins are screened for a predetermined property differ from the target protein. Also, claim 1 recites that the predetermined property is selected from among a chemical, physical and biological property of the target protein. The specification explains that the screening methods can be applied to any chosen feature of a target protein, including a chemical, physical and biological property (see for example, page 9 line 6 to page 10, line 3). The specification describes that exemplary biological properties can include, biological efficiency, transduction efficiency, gene/transgene expression, differential gene expression and induction activity, titer, progeny productivity, toxicity, cytotoxicity, immunogenicity, cell proliferation and/or differentiation

activity, anti-viral activity, morphogenetic activity, teratogenetic activity, pathogenetic activity, therapeutic activity, tumor suppressor activity, ontogenetic activity, oncogenetic activity, enzymatic activity, pharmacological activity, cell/tissue tropism and delivery (see for example, at page 10, lines 9-18). The specification also explains that such properties that are evolved by the methods include activities that may have nothing to do with natural activities of a protein, such as evolving a chemical or physical property, for example the evolution of a site that confers immunogenicity on a protein (see for example, at page 14, lines 1-7).

Applicant respectfully submits that the term "predetermined property" is definite as used in the claims and read in light of the instant specification. The claim specifies that the property includes chemical, physical and biological properties of a target protein. The specification provides a plethora of examples of such properties. One of skill in the art would understand that the methods as claimed are applicable to any predetermined property chosen to be evolved by the method. Claim 9 depends from claim 1; hence, the use of the term "predetermined property" finds antecedent basis and definition in claim 1. Therefore, Applicant respectfully submits that claims 1 and 9 are definite.

Claim 16 is allegedly indefinite because it recites "replacing each codon that is a hit with a codon encoding the remaining amino acids." It is alleged that it is unclear if such remaining amino acids are selected from a list of remaining amino acids, from amino acids within the protein or both.

Applicant has amended the language of claim 16 to clarify that the method includes replacing each codon that is a hit position with a codon encoding another amino acid. The method produces a set of nucleic acid molecules each differing by at least one codon. The specification explains that such amino acids can be selected from a list of all possible amino acids (see for example at page 35, lines 1-10). The specification provides an example that includes using a list of native amino acids (see for example, at page 50, lines 19-22). Hence, Applicant respectfully submits claim 16 is definite.

Claim 17 is alleged to be indefinite in reciting "optimized lead," because the term is considered vague. The instant specification explains that leads can be further optimized by generating proteins that have combinations of leads. Applicant has clarified claim 17 by adding the definition "where an optimized lead comprises two or more hit positions" into the claim language. An explanation of the production of optimized leads that contain 2 or more hit positions can be found in the specification at pages 34-35. Hence, the term optimized lead, as used herein is definite.

Claims 19-21, 25 and 26 are allegedly indefinite in reciting the term "effected." Applicant respectfully submits that the term "to effect" is a verb commonly used in the English language to mean "to accomplish," "to cause to come into being" (see Merriam Webster's Dictionary 10th Ed. (1993) page 367; provided herein). Hence, for example, the phrase in claim 19 "the recombining is effected by a method selected from..." means "the recombining is accomplished by one of the methods listed therein. Similarly, in claim 20, the phrase "the modifications are effected in a selected domain of the target protein" means that the method cause modifications to occur in a selected domain. Therefore, Applicant respectfully submits that "effected" is used as a verb with its ordinary meaning and the claim language is definite.

Claims 24 and 26 are alleged to be indefinite in reciting "at step (b) the titer of the viral vectors in each set of cells is assessed" because the claimed subject matter allegedly does not "use" this information.

Applicant respectfully submits that this step represents an embodiment of the screening methods described in the specification that includes the step of assessing viral titer so that the activities of the screened proteins can be compared (see for example, at page 4, lines 6-8). Claims 24-26 depend from claim 7, which is directed to the screening method of claim 1 where nucleic acid molecules comprise viral vectors; and the cells are eukaryotic cells that are transduced with the vectors. The screening method of claim 1 includes the step of individually screening the sets of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein. Step (b) includes introducing each set of nucleic acid molecules into host cells and expressing the encoded protein, wherein the host cells are present in an addressable array. Thus, in the context of claims 24-26, the nucleic acid viral vectors are introduced into host cells and the titer of the viral vectors in each set of cells is assessed. The titering is used as a step in comparing the proteins expressed from the viral vectors. Therefore, claims 24-26 describe one of the embodiments of the method for use with viral vectors. Applicant respectfully submits that one of skill in the art in light of the specification would understand the "use" of this step in the methods as claimed.

Claim 16 is allegedly indefinite because it recites the limitation "codon that is a hit" but that such phrase lacks antecedent basis in claims 1 and 9 from which claim 16 depends. Claim 16 is amended herein for clarity to recite the phrase "codon that is a hit position." This

phrase finds antecedent basis in claim 1 which recites "each hit contains a hit position." Therefore, claim 16 is definite.

IV. Rejections Under 35 U.S.C. §102

Claims 1-23, 27 and 42-44 are rejected under 35 U.S.C. §102(e) as being anticipated by Short (US Patent No. 6,171,820 B1). It is alleged that Short discloses a methods of producing a set of mutagenized progeny polynucleotides encoding a polypeptide from a parental template polynucleotide via codon site-saturation mutagenesis, wherein at each original codon there is produced at least one substitute codon encoding each of the 20 naturally occurring amino acids. It also is alleged that Short discloses the methods using plasmids and viral vectors in bacterial host cells in addressable arrays, analyzing kinetic activity as improved stability and optionally repeating the steps of the method.

This rejection is respectfully traversed.

RELEVANT LAW

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S., 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

Rejections under 35 U.S.C. §102 are proper only when the claimed subject matter is identically disclosed or described in the "prior art" . . .the [r]eference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings in the cited references. Such picking and choosing may be entirely proper when making a rejection of a §103, obviousness rejection,

where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the *similarity* of the subject matter which he claims to the prior art, but it has no place in the making of a §102, anticipation rejection." (Emphasis in original). In re Arkey, Eardly, and Long, 455 F.2d 586, 172 USPQ 524 (CCPA, 1972).

The Claims

Claim 1 is directed to a process for the identification of a peptide, polypeptide, or protein that differs in a predetermined property from a target protein. The method includes the steps of (a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, (b) introducing each set of nucleic acid molecules into host cells and expressing the encoded protein, wherein the host cells are present in an addressable array; and(c) individually screening the sets of encoded proteins, whereby one or more proteins that have a predetermined property that differs from the target protein are identified. The predetermined property is selected from the group consisting of a chemical, physical and biological property of the target protein. The identified proteins each are designated as a hit, where each hit contains a mutation designated a hit position. Dependent claims specify variations of the method including methods of designed and/or synthesizing nucleic acids, methods using addressable arrays, solid supports, types of nucleic acid molecules, variations in the nucleic acids, target proteins and predetermined properties used in the methods and addition steps that can be used with the methods.

The disclosure of Short

Short is directed to mutagenesis techniques for directed evolution of proteins. The patent describes a saturation mutagenesis method that includes generating a set of modified polypeptides in which a full range of amino acid substitutions is represented at each amino acid position. The method uses degenerate oligonucleotide cassettes to generate sets of modified polynucleotides encoding modified polypeptides. In the methods disclosed by Short, each reaction vessel contains at least 32 distinct polynucleotides encoding 20 distinct polypeptides (column 34, lines 43-49). The mixture of polynucleotides is transformed together into host cells and screened.

ANALYSIS

Short does not anticipate the methods as set forth in the instant claims. The claimed methods are directed to individually introducing and screening sets of nucleic acid molecules each encoding a modified protein in host cells. For example, claim 1 recites that each set of

nucleic acid molecules is introduced into host cells present in an addressable array. Thus, the sets of nucleic acids are introduced individually into host cells. Each set of nucleic acids encodes a modified form of a target protein and following introduction into host cells, these modified forms are expressed. The encoded proteins are individually screened in an addressable array and proteins that have a predetermined property that differs from the target protein are identified.

In contrast, the methods disclosed by Short are not directed to methods that include individually introducing and screening sets of nucleic acid molecules each encoding a modified protein. Short specifically states that the saturation mutagenesis described therein involves generating reaction vessels, each of which has 32 distinct progeny nucleotides. In addition, the 32 distinct progeny nucleotides in each reaction vessel encode 20 distinct polypeptides (column 34, lines 43-60). These mixtures of nucleic acids are amplified in a host (*E. coli*) together. They are not individually introduced into host cells in an addressable array.

Although the Examiner alleges that Short describes introducing mutagenized polynucleotides into host cells in an addressable array, Applicant respectfully submits that such statement is a misinterpretation of the description presented therein. Example 5 states that mutagenesis of a dehalogenase enzyme is performed in a reaction vessel with 32-fold degenerate primers (col. 55, lines 38-59). The mixture of polynucleotides generated with the degenerate primers is then used to transform *E. coli* (col. 55, lines 60-61). The entire transformation mix is plated on a large plate together (col. 55, lines 61-63). Although individual colonies are picked and grown in microtiter plates, this step occurs after the introduction of the polynucleotides as a mixture into host cells. Hence, unlike the instantly claimed methods, in Example 5, the individual sets of polynucleotides were not individually introduced into host cells in an addressable array. The sets of polynucleotides were mixed together as a result of the degenerate PCR in the single reaction vessel. Further, the mixture was transformed together as a mixture, not individually, into host *E. coli* cells. Thus, Example 5 fails to disclose any methods that individually introduce sets of nucleic acids into host cells in an addressable array.

Anticipation requires that a reference disclose each and every element of the claimed subject matter. Short fails to disclose any methods that include producing a population of sets of nucleic acid molecules that encode modified forms of a target protein where each set of nucleic acid molecules is introduced into host cells present in an addressable array. Thus,

this reference fails to disclose each and every element of the method of claim 1. Therefore, Short does not anticipate claim 1 or any claims dependent thereon, including claims 2-23, 27 and 42-44 named in this rejection.

* * *

In view of the above, reconsideration and allowance are respectfully requested

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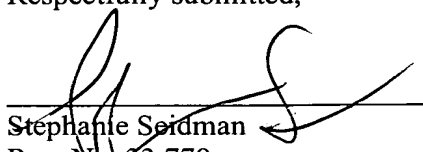
Respectfully submitted,



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chemically or otherwise identifiable, such as by an identifiable symbology, including a bar code, or can be color coded.

1. Generation of Diversity using a semi-rational approach

A semi-rational approach to creating diversity or evolving genes is provided herein. The goal is to create diversity but to decrease the number of molecules to be screened. By reducing the numbers, the molecules can be screened in high throughput format molecule-by-molecule (or groups thereof).

Generation of diversity at the nucleic level, in principle, can be accomplished by a number of diverse technologies like mutagenesis (either site-directed or random), recombination, shuffling and de-novo synthesis. These different technologies differ in the degree of diversity they generate as well as in the minimal length of the unitary change they can introduce (from single base to large domains). The outcome of step 1 is a collection of diverse, although highly related, molecules that constitutes what is called a 'library'.

This step is crucial, since it provides the initial conditions for the entire process and is determinative of the outcome. The chances of finding an optimized gene version in a library is a function of the total diversity present in the library. In addition, the type of diversity introduced (such as, but not limited to, single point mutations, multiple point mutations, scarce small rearrangements, recombination of large domains, multiple shuffling) condition the outcome, particularly with respect to the generation of new variants compared to the original gene, and the probability that the new variants, not only exhibit the "evolved" function or property, but also work in their natural biological networks where they are expected to act by interacting, recognizing, and/or being recognized, by a large panoply of other proteins and other molecules.

Rapid discovery of protein variants at the amino acid level by rational mutagenesis (aa-scan)

A method, referred to herein as an amino-acid scan method for directed evolution, is provided herein for generating protein variants. This method can be performed on an entire protein or selected domains thereof, or can be used to identify benchmark sequences, such as functional domains, and, for example, recombine them as exchangeable units or restrict the diversity to limited or specific regions of the protein. Not only can this method be used with the processes provided herein, but it also has applications for any methods that use such variants or require generation of such variants, such as, but not limited to, searches for consensus sequences and homology regions that are used in functional genomics, functional proteomics; comparative modeling in protein crystallography and protein modeling; searches for natural diversity, (*e.g.*, directed evolution methods in 6,171,820, 6,238,884, 6,174,673, 6,057,103, 6,001,574, 5,763,239,); exon- or family-shuffling-based diversity (*e.g.*, directed evolution using gene shuffling (see, *e.g.*, U.S. Patent Nos. 6,096,548, 6,117,679, 6,165,793, 6,180,406, 6,132,970); the optimization of only the CDRs regions (*e.g.*, directed evolution of antibodies see., *e.g.*, U.S. Patent Nos. 5,723,323, 6,258,530, 5770,434, 5,862,514) and other methods (see, *e.g.*, U.S. Patent Nos. 5,837,500, 5,571,698, 6,156,509).

The amino-acid scanning-based method provided herein has advantages that prior methods do not have. For example, prior methods are based upon the underlying assumption that there are parts of the molecule (gene or protein) that are sufficiently adapted to perform their respective function, and further changes are not advantageous. Such methods do not look at total potential plasticity of a given molecule, but at the plasticity still permitted while keeping some basic functions in place. By choosing this route, however, additional potential variation is missed. The potential in the intrinsic plasticity of those regions that are presumed 'preserved' is lost. For instance, methods (*e.g.*, those in U.S.